GENETIC VARIATION IN THE ENDEMIC CALIFORNIA SEDGE *CAREX HIRTISSIMA* (CYPERACEAE)

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ABSTRACT

Carex hirtissima is a perennial sedge endemic to montane forests in the foothills of the Sierra Nevada and the Coast Ranges in central California. In a study of four natural populations and all known herbarium specimens from 63 herbaria, I investigated patterns of variation in allozymes, structural features and chromosome numbers. Seventeen putative enzyme-coding loci were assayed and five were found to be polymorphic. Deviations from Hardy-Weinberg equilibrium and high positive fixation indices suggested that inbreeding is the dominant mating system. Cluster analysis of genetic distances calculated among populations showed that populations of *C. hirtissima* are quite similar and that this species is probably more closely related to *C. gynodynama* than to *C. mendocinensis,* two other species endemic to the California Floristic Province. Chromosome numbers were determined for *C. hirtissima* for the first time in this study and found to be $n = 35$ in one population and $n = 36$ in another. A new glabrous morph of *C. hirtissima* was found in one population, but both glabrous and pubescent morphs had the same chromosome number and similar allozyme frequencies.

Carex hirtissima Boott is a perennial sedge endemic to montane forests on the western slopes of the Sierra Nevada and in the Coast Ranges of central California. The species was described in 1880 by W. Boott based on a collection by A. Kellogg from Summit Camp, Bear Valley, in the Sierra Nevada. It has since been found in the foothills from Butte County south to Tuolumne County as well as on the western side of the Central Valley in the Coast Ranges (Fig. 1). *Carex hirtissima* is described as rare in Munz (1959).

The taxonomic placement of *C. hirtissima* within the genus has been problematic. Kükenthal (1909), in his world monograph of *Carex,* placed *C. hirtissima* in section *Hirtae,* while Mackenzie (in Abrams 1923) included it in section *Triquetrae* along with several other sedges having prominent pubescence on leaves, culms, and perigynia. Mackenzie (1935) later moved *C. hirtissima* to section *Sylvaticae* along with two other species endemic to the California Floristic Province, *C. gynodynama* and *C. mendocinensis,* and Munz (1959) followed this treatment.

In conjunction with a monographic study of *Carex* section *Hymenochlaenae,* I assayed allozyme variation, determined chromo-

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TABLE 1. SAMPLING SITES FOR *CAREX HIRTISSIMA*. All collection numbers are those of the author. Sample size for allozyme analysis is given in parentheses after each location.

Challenge (3564): Yuba Co., Challenge Cut-Off Road, 2 mi S of jct. with Forbestown Rd. 39°29′N, 121°16′W. (24)
Forbestown (3068/3069/3566): Butte Co., Plumas National Forest, 2 mi W of Forbestown along Ponderosa Way. 39°31′N, 121°19′W. (29)
Georgetown (3075/3567): Eldorado Co., 1 mi S of Georgetown on the W side of Hwy. 193. 38°54′N, 120°50′W. (11)
Round Burn (3580): Lake Co., ca. 2 mi S of the Elk Mountain Ranger Station, Mendocino National Forest. 39°16′N, 122°56′W. (20)

some numbers, and measured structural characters of *C. hirtissima* to determine patterns of variation within and among populations and to evaluate its relationship with *C. mendocinensis* and *C. gynodynama*. In this paper, I present the results of these studies and compare levels of genetic variation in *C. hirtissima* to those in other endemic *Carex* species.

METHODS

Field sampling. Four natural populations (Table 1) were studied in the field during the flowering season in March 1986. At each site, soil pH was determined colorimetrically using a soil pH test kit obtained from the Cornell Agronomy Department. Leaf samples for allozyme analysis were collected from randomly selected individuals at each site with sample sizes ranging from 11 to 29, depending on the size of each natural population. Voucher specimens, including flowering shoots where possible, were pressed from each sampled plant and are deposited at MTMG.

Chromosomes. At the Challenge and Forbestown sites, young staminate spikes just emerging from their sheaths were also collected from some of the same individuals to determine chromosome numbers. Sample size was limited by the number of plants at the correct developmental stage—all available were collected. Young staminate spikes were fixed in modified Carnoy's solution (6:3:1, ethanol: chloroform: propionic acid), stained using alcoholic hydrochloric acid-carmine (Snow 1963), and squashed in Hoyer's mounting medium (Radford et al. 1974). At least six cells were counted per individual. Voucher specimens are deposited at MTMG.

Allozyme analysis. Field-collected young shoots were stored in plastic bags at 4°C for up to one month prior to electrophoresis. No differences in staining activity were noted between fresh and refrigerated material except for alcohol dehydrogenase which stained more intensely when refrigerated material was used. Standard tech-

niques of horizontal starch gel electrophoresis of soluble enzymes were used to separate the electromorphs (Shields et al. 1983; Wendel and Weeden 1989) as previously described (Waterway 1990, 1994). Ten soluble enzymes were assayed: alcohol dehydrogenase (ADH, E.C. 1.1.1.1), glucosephosphate isomerase (GPI, E.C. 5.3.1.9), aspartate aminotransferase (AAT, E.C. 2.6.1.1), acid phosphatasae (ACP, E.C. 3.1.3.2), and triosephosphate isomerase (TPI, E.C. 5.3.1.1) using a lithium-borate/tris-citrate discontinuous buffer system at pH 8.3, and phosphoglucomutase (PGM, E.C. 5.4.2.2), malic dehydrogenase (MDH, E.C. 1.1.1.37), 6-phosphogluconate dehydrogenase (6PGD, E.C. 1.1.1.44), menadione reductase (MDR, E.C. 1.6.99.3), and NADH diaphorase (DIA, E.C. 1.8.1.4) using a histidine buffer system at pH 6.5.

The genetic basis for the observed banding patterns was inferred from comparison of pollen extracts and leaf tissue extracts (cf. Weeden and Gottlieb 1979) and known subunit composition and numbers of isozymes usually found in diploid angiosperms (Weeden and Wendel 1989). Putative loci and alleles were numbered and lettered, respectively, starting with those migrating the fastest. Naming of loci and alleles was identical to that in Waterway (1990), allowing direct comparison with related species in California.

Allele frequencies and average heterozygosities were calculated for each population and for the species overall. BIOSYS-1 (Swofford and Selander 1981) was used to calculate the mean expected heterozygosity under Hardy-Weinberg equilibrium, to test for deviations from Hardy-Weinberg equilibrium, and to calculate Wright's (1965) fixation index. Nei's gene diversity statistics H_T, H_S, and G_{ST}, unbiased for sample size (Nei and Chesser 1983) were calculated using GENESTAT (Whitkus 1985). Nei's (1972) genetic distance coefficients as modified by Sattler and Hilburn (1985) and U statistics (Mueller and Ayala 1982) to test for significant differences among populations and between *C. hirtissima* and two related species, *C. gynodynama* and *C. mendocinensis,* were calculated using the program SIDGEND (Sattler and Hilburn 1985). Relationships among populations and species based on the allozyme data were determined by clustering the genetic distance coefficients using an unweighted pair group means analysis (UPGMA, Sneath and Sokal 1973).

Morphology. Observations on the field-collected specimens described above and from those collected at three of the sites in April, 1984 were supplemented by examination of herbarium specimens borrowed from BH, CAS, CHSC, DAV, DS, GH, MIN, MICH, NY, NYS, ROPA, UC, and US (abbreviations following Holmgren et al. 1990). Loans were initially requested from 63 North American herbaria, but only the 13 listed here had any specimens of *C. hirtissima.*

To assess variability within and among populations, the characters listed in Waterway (1990) were measured on 50 specimens of *C. hirtissima* representing 13 different populations. Principal components analysis (SAS Institute, Inc. 1985, procedure PRINCOMP) based on the 23 quantitative characters for which data were available for most specimens (Table 6, all characters except number 4) was used to visualize the pattern of structural variation within *C. hirtissima.* For comparison with the related species, *C. gynodynama* and *C. mendocinensis,* a canonical discriminant analysis (SAS Institute, Inc. 1985, procedure CANDISC) in which the three species were considered statistical populations was calculated based on the same 23 characters measured from herbarium specimens. Each specimen was classified into one of the three species based on qualitative characters such as color and pubescence and these characters were not included in the canonical analysis. Log transformations were used in both analyses.

Results

Habitat and geographic distribution. Carex hirtissima grows in openings in *Pinus ponderosa* forests at lower elevations on the western slopes of the Sierra Nevada from Tuolumne Co. north to Butte Co. It also occurs in similar habitats, but more rarely, in the Coast Ranges of Lake and Mendocino counties (Fig. 1). Twenty-four different collections representing 15 sites were found in a survey of specimens from 63 herbaria. The four populations sampled in this study were growing in soils with pH ranging from 7.4 to 7.6 and all were in seepage areas or along streams. Three of the four sites I sampled were in areas that had been clearcut within the last few years. The fourth site was a small wet meadow in a residential area.

Variation in chromosome number. The two populations of *C. hirtissima* from which chromosome counts could be obtained were each characterized by a different haploid number. Three individuals from the Challenge population all had haploid numbers of 35 while three plants from the Forbestown population each had $n = 36$ (Fig. 2). Normal meiotic pairing was evident in all cells examined.

Allozyme variation. Seventeen putative loci were scored from the ten enzyme systems assayed. All isozymes were interpreted as dimeric enzymes except for PGM and DIA which were monomeric and MDR which was tetrameric. Although the plants had relatively high chromosome numbers suggesting possible polyploidy, the numbers of isozymes observed were those normally found in diploid plants. The four populations were monomorphic for all loci except the five listed in Table 2 (*Aat-3, Pgm-1, Pgm-2, 6-Pgd,* and *Mdh-4*). The smallest population (Georgetown) was monomorphic for the

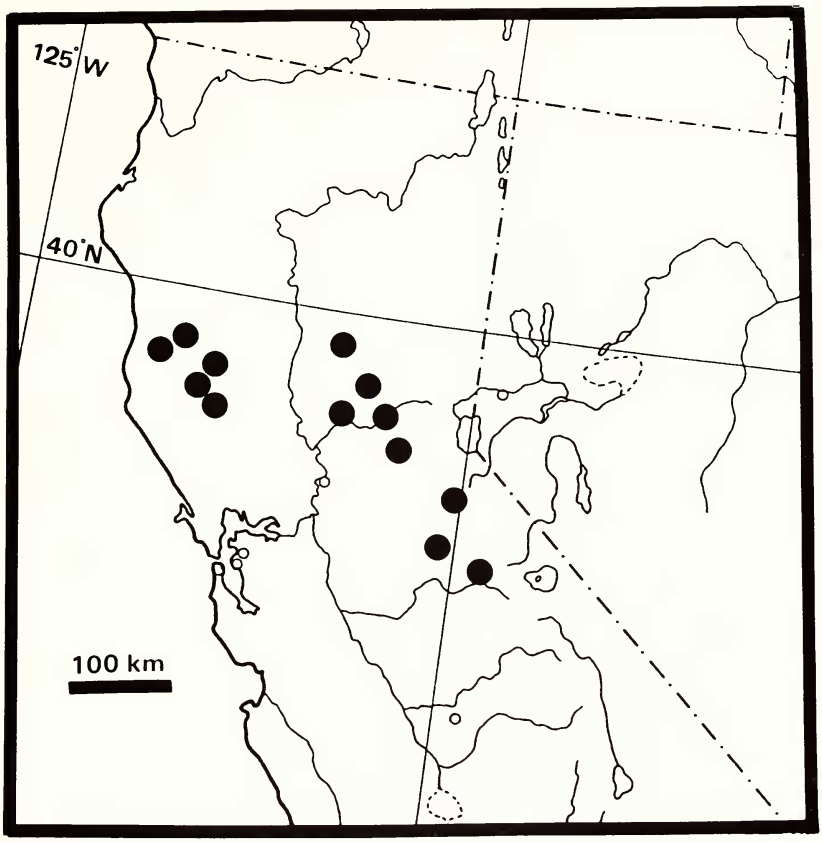

FIG. 1. Geographic distribution of *Carex hirtissima* in California.

most common allele at all loci sampled. *Carex hirtissima* shared many alleles with the closely related species *C. gynodynama* and *C. mendocinensis* (Waterway 1990). It was monomorphic for the most common allele found in both *C. gynodynama* and *C. mendocinensis* at *Gpi-1, Gpi-2, Dia-2, Tpi-1, Tpi-2, Acp-1, Mdh-2, Mdh-3,* and *Adh-1.* At *Mdr-1* and *Mdh-1, C. hirtissima* was monomorphic for the most common allele found in *C. gynodynama* and at *Aat-2,* monomorphic for the most common allele found in *C. mendocinensis.*

Measures of polymorphism and heterozygosity were low for *C. hirtissima* (Table 3). Mean number of alleles per locus ranged from 1.0 to 1.2 per population and no locus had more than 2 alleles. Five of the 17 loci assayed were polymorphic within the species (29.5%) and the percentage of polymorphic loci per population ranged from 0 to 23.5 percent. Expected heterozygosity was very low, with pop-

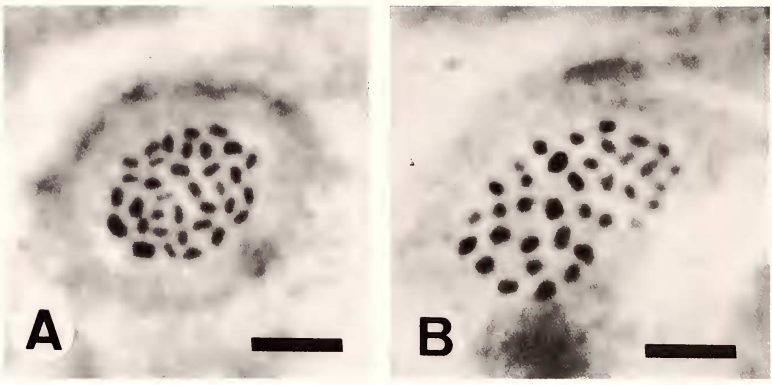

FIG. 2. *Carex hirtissima* chromosomes at metaphase I of meiosis. A) Georgetown population (n = 35); B) Forbestown population (n = 36).

ulations ranging from 0.03 to 0.07, while observed heterozygosity was even lower with values from 0 to 0.015. Allele frequencies differed significantly from those expected under Hardy-Weinberg equilibrium for all polymorphic loci within the populations. In each case, fixation indices were positive, indicating a deficiency of heterozygotes (Table 4). Nei's gene diversity statistics calculated from all 17 loci sampled were also very low for *C. hirtissima.* Values of H_S were less than 0.2 for each locus indicating low levels of variability within each local population. In contrast, values of G_{ST}, which measures the relative amount of diversity that can be apportioned among populations, were greater than 0.5 for the two most variable isozymes and averaged 0.361 over the polymorphic loci.

Genetic identities based on pairwise comparisons between populations averaged 0.954 ± 0.040 within *C. hirtissima,* higher than

TABLE 2. ALLOZYME FREQUENCIES AT POLYMORPHIC LOCI IN *CAREX HIRTISSIMA.*

Locus allele		Population			
		Challenge	Forbestown	Georgetown	Round Burn
Aat-3	*a*	0	0	0	0.05
	b	1	1	1	0.95
Pgm-1	*a*	0.78	0.93	1	0.78
	b	0.22	0.07	0	0.22
Pgm-2	*a*	0.73	0.86	0	1
	b	0.27	0.14	1	0
Mdh-4	*b*	0.04	0	0	0
	d	0.96	1	1	1
6-Pgd	*a*	0.16	0.07	0	0.80
	c	0.84	0.93	1	0.20

TABLE 3. GENETIC VARIABILITY MEASURES FOR THE FOUR SAMPLED POPULATIONS OF *CAREX HIRTISSIMA*. P = percentage of loci polymorphic; A = mean number of alleles per locus; H_{OBS} = observed heterozygosity; H_{EXP} = expected heterozygosity under Hardy-Weinberg equilibrium.

Population	P	A	H_{OBS}	H_{EXP}
Challenge	23.5	1.2	0.009	0.066
Forbestown	17.6	1.2	0.003	0.030
Georgetown	0	1.0	0.000	0.000
Round Burn	17.6	1.2	0.015	0.046
Overall	29.5	1.3	0.007	0.064

those comparing *C. hirtissima* with either *C. gynodynama* or *C. mendocinensis* (Table 5). A cluster analysis of these genetic distances showed the two populations from *Pinus ponderosa* forests in the Forbestown area to be the most similar, with the population from a *Pinus ponderosa/Pinus lambertiana* forest in the Coast Range somewhat differentiated, and the population from a wet meadow in the Sierra foothills to be the most differentiated (Fig. 3). The cluster analysis also suggested that *C. hirtissima* is more similar to *C. gynodynama* than to *C. mendocinensis*. The U statistics indicated significant differences between *C. hirtissima* and *C. gynodynama* at the 0.1 level, and significant differences between *C. hirtissima* and *C. mendocinensis* at the 0.05 level (Table 5).

Variation in morphology. Carex hirtissima is similar to *C. gynodynama* in having pilose leaves, culms, and perigynia, but differs from it by having longer and narrower leaves and perigynia and scales that are green to golden rather than dark purple as in *C. gynodynama*. Populations of *C. hirtissima* in the foothills of the Sierra Nevada tend to have more densely pilose leaves and culms than those in the Coast Ranges. The pubescence on the perigynia is more consistent from population to population than that on the leaves and culms. I discovered a previously undescribed form at the Forbestown site in Butte Co., CA (3566). The leaves, culms and perigynia of these plants were completely glabrous. Approximately one-fifth

TABLE 4. FIXATION INDICES FOR POLYMORPHIC LOCI IN POLYMORPHIC POPULATIONS OF *CAREX HIRTISSIMA*.

	Population		
Locus	Challenge	Forbestown	Round Burn
Aat-3	—	—	1.000
Pgm-1	0.679	0.641	0.570
Pgm-2	0.895	1.000	—
Mdh-4	1.000	—	—
Pgd-1	1.000	1.000	0.688

TABLE 5. MEAN GENETIC DISTANCES AND U STATISTICS BETWEEN SPECIES. Mean genetic distances calculated according to Sattler and Hilburn (1985) based on all pairwise comparisons among populations are given in the lower left triangle. U statistics (see text) are given in the upper right triangle. † denotes significant differences between taxa at the 0.1 level, * at the 0.05 level.

Species	n	*C. hirtissima*	*C. gynodynama*	*C. mendocinensis*
C. hirtissima	4	0.046 ± 0.040	0.135 ± 0.092†	0.266 ± 0.135*
C. gynodynama	5	0.152 ± 0.093	0.000 ± 0.000	0.197 ± 0.112†
C. mendocinensis	3	0.323 ± 0.149	0.216 ± 0.118	0.081 ± 0.040

of the population at this site belonged to this glabrous morph. This character did not correlate with any other structural difference. In addition, both glabrous and pubescent morphs had the same chromosome number and similar allozyme frequencies. Glabrous individuals tended to occur in clusters within the population, but these clusters were found throughout the population. These observations suggest that the pubescence is probably under simple genetic control, possibly regulated by a single gene or gene complex. None of the herbarium specimens I examined included any glabrous individuals. It is possible that this form occurs in other populations but has

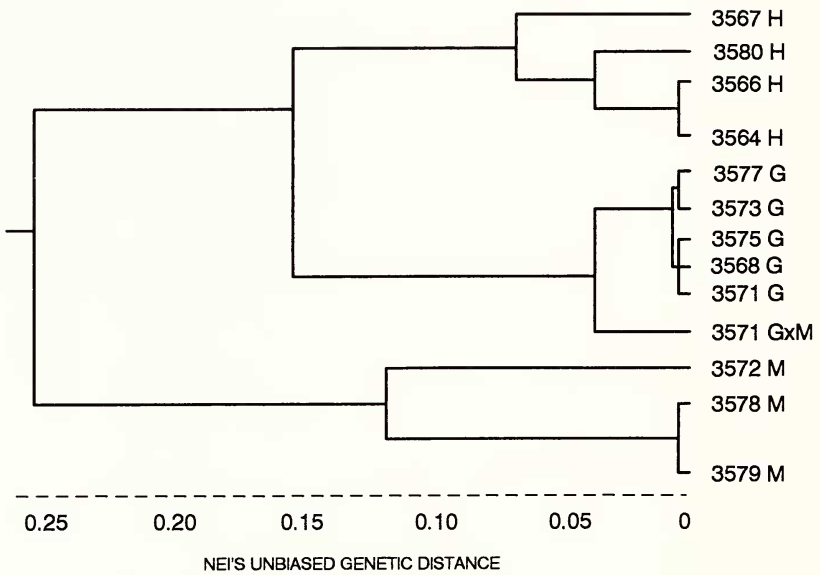

FIG. 3. Cluster analysis (UPGMA) of Nei's genetic distance coefficients as modified by Sattler and Hilburn (1985) based on allozyme frequency data from populations of *Carex hirtissima* (H), *C. gynodynama* (G), and *C. mendocinensis* (M). Population numbers correspond to Table 1 for *C. hirtissima* and to Waterway (1990) for the other species.

TABLE 6. MEANS, STANDARD DEVIATIONS, AND RANGES OF THE 24 MORPHOLOGICAL CHARACTERS MEASURED ON 50 HERBARIUM SPECIMENS OF *CAREX HIRTISSIMA*. All measurements are in mm except number 15 which is a ratio.

Character	Mean	SD	Range
1. Staminate spike length	18.2	3.8	10–27
2. Staminate spike width	3.6	0.9	2.2–6.0
3. Staminate peduncle length	5.6	5.6	0.1–25
4. Bract length, staminate spike	4.3	1.3	2–7
5. Upper pistillate spike length	11.7	3.9	5–22
6. Upper pistillate spike width	4.4	1.1	2–7
7. Bract length, upper pistillate spike	7.9	5.9	1.3–25
8. Lowest pistillate spike length	20.2	4.2	12–30
9. Lowest pistillate spike width	4.6	0.9	2–6
10. Lowest pistillate peduncle length	9.6	15.0	0.1–98
11. Bract blade length, lowest pistillate spike	55.3	31.5	4–137
12. Bract sheath length, lowest pistillate spike	15.6	15.6	0.5–87
13. Perigynium length	3.6	0.5	2.5–4.2
14. Perigynium width	1.5	0.2	1.0–1.8
15. Perigynium shape (distance from base to widest point/length)	0.5	0.1	0.3–0.9
16. Perigynium beak length	0.7	0.3	0.2–1.9
17. Pistillate scale length	2.9	0.5	2.0–4.1
18. Pistillate scale awn length	0.5	0.3	0.1–1.3
19. Achene length	2.5	0.3	2.0–3.1
20. Achene width	1.3	0.2	1.0–1.7
21. Length of stipe at base of achene	0.2	0.1	0.1–0.6
22. Widest basal leaf, width	6.0	2.1	2.7–12.0
23. Widest basal leaf, length	240.1	76.4	110–430
24. Length of lowest bladeless sheath	15.9	6.9	4–40

not been collected; it is also possible that glabrous individuals have been identified and filed as other usually glabrous species and were therefore not included in the loaned material examined in this study. Additional field observations are needed to determine the distribution of this glabrous form.

Scatter plots of various combinations of the quantitative characters did not reveal any geographic trends in morphology. Nor were geographic patterns apparent from the principal components analysis (not shown) based on the quantitative characters. Individuals collected from both sides of the Central Valley and from different elevations in the Sierra Nevada were intermixed on the graph of the first three principal components describing the structural variation.

In addition to the color differences noted above, *Carex hirtissima* can be distinguished from the related species *C. gynodynama* and *C. mendocinensis* based on the sizes of the pistillate and staminate spikes and peduncles, the perigynia, and the basal leaves (cf. Table 6 and Waterway 1990, table 3). Staminate spikes are comparable in size for *C. hirtissima* and *C. gynodynama* but longer and narrower in *C. mendocinensis*. However, the staminate spikes are usually lon-

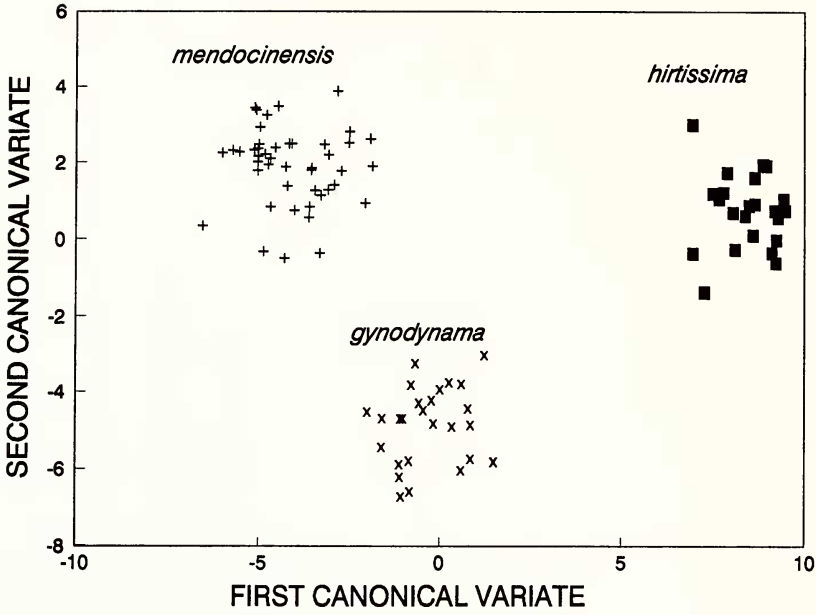

FIG. 4. Canonical discriminant analysis of structural data measured on herbarium specimens. Individuals of *C. gynodynama* (×), *C. mendocinensis* (+), and *C. hirtissima* (squares) are positioned according to their first and second canonical scores.

ger peduncled in *C. hirtissima* than in *C. gynodynama,* although the peduncles vary from about 1 mm to nearly 25 mm. Pistillate spikes of *C. hirtissima* vary from 0.5 to 3 cm in length and are shorter and narrower than those of *C. gynodynama* or *C. mendocinensis.* Basal leaves are generally much longer in *C. hirtissima* than in *C. gynodynama* or *C. mendocinensis.* Leaf widths vary from 2.7 to 12 mm, with a mean of 6.0 mm in *C. hirtissima,* narrower than the mean leaf width of 8.2 mm found in *C. gynodynama* and wider than the mean leaf width of 3.7 mm found in *C. mendocinensis.* The mean perigynium length in *C. hirtissima* was 3.6 mm in comparison to a mean length of 4.3 mm for *C. gynodynama* and 3.8 for *C. mendocinensis.*

The graph of the canonical discriminant scores (Fig. 4) illustrates the clear separation among the three species. Characters most heavily weighted on the first axis, which separates *C. hirtissima* from *C. mendocinensis* were the length of the basal leaves, the length and width of the staminate spikes, the length of the lowest staminate spike, and the length of the achene. On the second axis, which separates *C. hirtissima* from *C. gynodynama,* the most heavily weighted characters were the length and width of the basal leaves,

the length of the staminate peduncle, the lengths of the pistillate spikes, and the length of the perigynium.

Discussion

The amount of genetic variation within plant populations and within species may be influenced by many factors. Not surprisingly, the mating system has been shown to be one of the most important influences, with inbreeders having significantly lower levels of polymorphism and heterozygosity within populations and greater differentiation among populations than outcrossers (Hamrick and Godt 1989; Hamrick 1991). The allozyme data suggest that inbreeding is prevalent in *C. hirtissima.* Levels of heterozygosity were very low and values for all polymorphic loci showed significant deviations from Hardy-Weinberg equilibrium with positive fixation indices indicating a deficiency of heterozygotes. The rare alleles were found as homozygotes rather than as heterozygotes, suggesting that outcrossing is not very common. The inflorescence morphology with staminate spikes above, but close to, the pistillate ones, and the synchronous flowering phenology suggest that a high rate of selfing is likely. Substructuring within populations may also be an indication that inbreeding is common. The fact that the glabrous individuals in the Forbestown population were found in clusters rather than randomly throughout the population suggests that seeds do not disperse very far and mating occurs among these closely related individuals.

Differentiation among populations is also apparent in *C. hirtissima.* G_{ST} values for the polymorphic loci indicated that about 36% of the allozyme variation was apportioned among populations. Furthermore, chromosome number also appears to differ among populations. Chromosome numbers obtained from three individuals in each of two populations were the same within populations but different between them. Different chromosome numbers in different populations have also been noted in other *Carex* species (Waterway 1990; Hoshino 1992; Hoshino and Waterway 1994), and crosses between different chromosome races within a species do not necessarily show any reduced fertility in *Carex* (Whitkus 1988). As noted above, several structural features also vary both within and among populations. Genetic distance was not correlated with geographic distance as the populations of *C. hirtissima* from the foothills of the Sierra Nevada were quite similar to the one from the Coast Ranges. One rare allele was found only at Round Burn and two others were found only in the Forbestown area. In the cluster analysis, the two populations from the Forbestown area clustered more closely to the population from Round Burn in Lake Co. than to the Georgetown population. This disjunct distribution between

the Coast Ranges and the Sierra Nevada is quite common (Howell 1946) suggesting that transport of propagules from one area to another may occur quite frequently or that the disjunction is relatively recent. The Georgetown population was most distinct from the others, both in habitat and in allozyme frequencies. This population was in a small, isolated wet meadow and was monomorphic for all allozyme loci assayed, suggesting the possibility of a founder effect or a genetic bottleneck.

Levels of genetic variation and the pattern of differentiation among populations in *C. hirtissima* are quite comparable to those found in another rare sedge, *C. misera,* which is endemic to the southern Appalachians (Schell and Waterway 1992). Another related California endemic, *C. gynodynama,* has even lower levels of polymorphism and heterozygosity within populations than either *C. hirtissima* or *C. misera* (Waterway 1990). In all three cases, inbreeding is probably the primary mating system. While low levels of variation in these endemic species may be a cause for concern about their evolutionary potential and therefore their persistence, the amount of allozyme variation is comparable to that of some more widespread inbreeding *Carex* species (reviewed in Schell and Waterway 1992). An important consideration for the conservation of species such as *C. hirtissima* is the genetic differentiation among populations and the resulting need for conservation of many populations to conserve the genetic diversity. In this study, most of the populations sampled were found in openings in the forest, often associated with logging activities. Disturbance events such as this may be important in creating habitat suitable for the germination and/or growth of *C. hirtissima.* Since *C. hirtissima* has been collected from only 15 localities, its conservation status should be considered. Additional field sampling is required to determine if this small number of sites reflects its actual abundance and geographic distribution. Further search should also be made for the glabrous form, thus far found only in the Forbestown area.

The allozyme data gathered in this study also support the classification of *C. hirtissima* in the same section with *C. gynodynama* and *C. mendocinensis.* The cluster analysis (Fig. 3) and the similarities in structural features (Table 6, Fig. 4) suggest a close relationship to *C. gynodynama.* The chromosome numbers of *C. hirtissima* at $n = 35$ or 36 are higher than any other species classified by Mackenzie into section *Sylvaticae* (Waterway 1988) and closer to *C. mendocinensis* ($n = 28$, 29, or 30) than to *C. gynodynama* ($n = 25$ or 26). However, aneuploid sequences within sections and within species of *Carex* are common, in part due to the holocentric chromosomes (Wahl 1940; Hoshino and Waterway 1994).

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Literature Cited

Abrams, L. 1923. Illustrated flora of the Pacific states. Vol. 1. Ophioglossaceae to Aristolochiaceae. Stanford University Press, Stanford, CA.

Hamrick, J. L. and M. J. W. Godt. 1989. Allozyme diversity in plant species. Pp. 43–63 *in* A. H. D. Brown, M. T. Clegg, A. L. Kahler, and B. S. Weir (eds.), Plant population genetics, breeding, and genetic resources. Sinauer, Sunderland, MA.

Hamrick, J. L., M. J. W. Godt, D. A. Murawski, and M. D. Loveless. 1991. Correlations between species traits and allozyme diversity: implications for conservation biology. Pp. 31–44 *in* D. A. Falk and K. E. Holsinger (eds.), Genetics and conservation of rare plants. Oxford University Press, New York.

Holmgren, P. K., N. H. Holmgren, and L. C. Barnett. 1990. Index herbariorum, part 1, The herbaria of the world, 8th ed. New York Botanical Garden, New York.

Howell, J. T. 1946. On the botany of Bartlett Mountain. Leaflets of Western Botany 4:254–258.

Hoshino, T. 1992. Cytogeographical study of four aneuploids of *Carex oxyandra* Kudo in Japan. Botanical Magazine, Tokyo 105:639–648.

Hoshino, T. and M. J. Waterway. 1994. Cytogeography and meiotic chromosome configurations of six intraspecific aneuploids of *Carex conica* Boott (Cyperaceae) in Japan. Joural of Plant Research 107:131–138.

Kükenthal, G. 1909. Cyperaceae: Caricoideae. Das Pflanzenreich 4(20):1–824. Engelmann, Leipzig.

Mackenzie, K. K. 1935. Cyperaceae—Cariceae. Pp. 169–478 *in* North American Flora, Vol. 18. New York Botanical Garden, New York.

Mueller, L. D. and F. J. Ayala. 1982. Estimation and interpretation of genetic distances in empirical studies. Genetical Research 40:127–137.

Munz, P. A. 1959. A California flora. University of California Press, Berkeley, CA.

Nei, M. 1972. Genetic distance between populations. American Naturalist 106:283–292.

Nei, M. and R. K. Chesser. 1983. Estimation of fixation indices and gene diversities. Annals Human Genetics 47:253–259.

Radford, A. E., W. C. Dickison, J. R. Massey, and C. R. Bell. 1974. Vascular plant systematics. Harper and Row, New York.

SAS Institute, Inc. 1985. SAS/STAT guide for personal computers, version 6. SAS Institute, Inc., Cary, NC.

Sattler, P. W. and L. R. Hilburn. 1985. A program for calculating genetic distance, and its use in determining significant differences in genetic similarity between two groups of populations. Journal of Heredity 76:400.

Schell, C. M. and M. J. Waterway. 1992. Allozyme variation and the genetic structure of populations of the rare sedge *Carex misera* (Cyperaceae). Plant Species Biology 7:141–150.

Shields, C. R., T. J. Orton, and C. W. Stuber. 1983. An outline of general resource needs and procedures for the electrophoretic separation of active enzymes from plant tissues. Pp. 443–468 *in* S. D. Tanksley, and T. J. Orton (eds.), Isozymes in plant genetics and breeding. Elsevier, Amsterdam.

Sneath, P. H. and R. R. Sokal. 1973. Numerical taxonomy: the principles and practice of numerical classification. W. H. Freeman, San Francisco.

Snow, R. 1963. Alcoholic hydrochloric acid-carmine as a stain for chromosomes in squash preparations. Stain Technology 38:9–13.
Swofford, D. L. and R. B. Selander. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. Journal of Heredity 72:281–283.
Wahl, H. A. 1940. Chromosome numbers and meiosis in the genus *Carex*. American Journal of Botany 27:458–470.
Waterway, M. J. 1988. Systematic studies in *Carex* section *Hymenochlaenae* Drejer (Cyperaceae). Ph.D. Dissertation. Cornell University, Ithaca, NY.
Waterway, M. J. 1990. Genetic differentiation and hybridization between *Carex gynodynama* and *C. mendocinensis* (Cyperaceae) in California. American Journal of Botany 77:826–838.
Waterway, M. J. 1994. Evidence for the hybrid origin of *Carex knieskernii* (Cyperaceae) with comments on hybridization in the genus *Carex*. Canadian Journal of Botany 72:860–871.
Weeden, N. F. and L. D. Gottlieb. 1979. Distinguishing allozymes and isozymes of phospho-glucoisomerases by electrophoretic comparisons of pollen and somatic tissues. Biochemical Genetics 17:287–296.
Weeden, N. F. and J. F. Wendel. 1989. Genetics of plant isozymes. Pp. 46–72 *in* D. E. Soltis and P. S. Soltis (eds.), Isozymes in plant biology. Dioscorides Press, Portland, OR.
Wendel, J. F. and N. F. Weeden. 1989. Visualization and interpretation of plant isozymes. Pp. 5–45 *in* D. E. Soltis and P. S. Soltis (eds.), Isozymes in plant biology. Dioscorides Press, Portland, OR.
Whitkus, R. 1985. FORTRAN program for computing genetic statistics from allele frequency data. Journal of Heredity 76:142.
Wright, S. 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 19:395–420.

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NOTEWORTHY COLLECTIONS

Baja California Sur

Acalypha ostryifolia Ridd. (Euphorbiaceae).—Baja California Sur, 15 km al E de La Paz, Presa "La Buena Mujer," 24°13′N, 110°15′W, 370 m, matorral sarcocaule, ladera, 13 Oct 1987, *Domínguez 552* (RSA).

Previous knowledge. Eastern United States to Arizona through much of mainland México to Central America.

Significance. First record from the Baja California Peninsula. This species can be readily separated from the other Baja Californian *Acalypha* by the combination of an annual habit and deeply divided pistillate bracts.

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